PART-A

ELECTROCHEMICAL STUDIES OF SODIUM LEVOTHYROXINE AT SURFACTANT MODIFIED CARBON PASTE ELECTRODE


Sodium levothyroxine
3.1. Introduction

In the present chapter the electrochemical response of sodium levothyroxine (T₄) at carbon paste electrode in the presence of 0.1M HCl as supporting electrolyte was investigated by the cyclic voltammetry. The discussion involves the chemistry, biological relevance of sodium levothyroxine and its oxidation behavior in 0.1 M HCl solution at the bare and surfactant modified carbon paste electrode. It showed a well-defined oxidation peak and two sensitive and indiscernible reduction peaks at the bare carbon paste electrode. The effect of concentration and scan rate of sodium levothyroxine was studied. The scan rate effect showed that the electrode process is adsorption controlled. The effect of surfactants like sodium dodecyl sulphate (SDS), cetyltrimethylammonium bromide (CTAB), and tritonX-100 (TX-100) were studied by mobilization and immobilization methods. The concentration effect of all the three surfactants was studied. Among these SDS showed excellent enhancement for both oxidation peak and reduction peak currents. Thus this method offers a promising, relatively uncomplicated protocol for the preparation of biosensors.

3.2. Chemistry of Sodium Levothyroxine

Sodium Levothyroxine (Scheme 3.1), also L-thyroxine, synthetic T₄, or 3, 5, 3', 5'-tetraiodo-L-thyronine, is a synthetic form of thyroxine (thyroid hormone), used as a hormone replacement for patients with thyroid problems. The natural hormone is chemically in the chiral L-form, as is the pharmaceutical agent. T₄, a form of thyroid hormones, is the major hormone secreted by the follicular cells of the thyroid gland. Thyroxine was first isolated in pure form in 1914 at the Mayo Clinic by Edward Calvin Kendall from extracts of hog thyroid glands [1]. The hormone was synthesized in 1927 by British chemists Charles Robert Harington and George Barger. T₄ is involved in controlling the rate of metabolic processes in the body and influencing physical development. Administration of thyroxine has been shown to significantly increase the concentration of nerve growth factor in the brains. Thyroxine is a prohormone and a reservoir for the active thyroid hormone triiodothyronine (T₃), which is about four times more potent. T₄ is converted in the tissues by deiodinases, including thyroid hormone iodine peroxidase (TPO), to T₃. The "D" isomer is called "Dextrothyroxine" and is used as a lipid modifying agent [2].
3.2.1. Biosynthesis of Sodium Levothyroxine

Thyroxine is synthesized via the iodination and covalent bonding of the phenyl portions of tyrosine residues found in an initial peptide, thyroglobulin, which is secreted into thyroid granules. These iodinated diphenyl compounds are cleaved from their peptide backbone upon being stimulated by thyroid-stimulating hormone (TSH). T4 is transported in blood, with 99.95% of the secreted T4 being protein-bound, principally to thyroxine-binding globulin (TBG), and, to a lesser extent, to transthyretin and serum albumin. The half-life of thyroxine once released into the blood circulatory system is about 1 week. Thyroxine can be measured as free thyroxine, which is an indicator of thyroxine activity in the body. It can also be measured as total thyroxine, which also depends on the thyroxine that is bound to TBG. A related parameter is the free thyroxine index, which is total thyroxine multiplied by thyroid hormone uptake, which, in turn, is a measure of the unbound thyroxine binding globulins. The normal human adult range of T4 in blood is 4 - 11 μg/dL [3].

3.2.2. Biological Relevance of Sodium Levothyroxine

Thyroxine (T4) is an important biological component produced in the thyroid glands. The practical significances of thyroxine measurements for the diagnosis of hyperthyroidism and hypothyroidism have been known for many years. Levothyroxine is typically used to treat hypothyroidism [4]. It may also be used to treat goiter via its ability to lower thyroid-stimulating hormone (TSH), a hormone that is considered goiter-inducing [5, 6]. Dosing must be carefully controlled to achieve TSH levels within the normal reference range. Long-term suppression of TSH values below normal values will frequently cause cardiac side-effects and contribute to decreases in bone mineral density (high TSH levels are also well known to contribute to osteoporosis) [7].

Patients prescribed too high a dose of levothyroxine may experience effects that mimic hyperthyroidism. Overdose can result in heart palpitations, abdominal pain, nausea, anxiousness, confusion, agitation, insomnia, weight loss, and increased appetite. Allergic reactions to the drug are characterized by symptoms such as difficulty breathing, shortness of breath, or swelling of the face and tongue. Acute overdose may cause fever, hypoglycemia, heart failure, coma and unrecognized adrenal insufficiency. Acute massive overdose may be life-threatening; treatment
should be symptomatic and supportive. Massive overdose may require beta-blockers for increased sympathomimetic activity [8, 9]. Foods and other substances interfere with absorption of thyroxine for example calcium and iron supplements and soy products can reduce absorption of the drug. Grapefruit juice may delay the absorption of levothyroxine, Other substances that reduce absorption are aluminium and magnesium containing antacids, simethicone or sucralfate, cholestyramine, colestipol, Kayexalate. A study suggests that coffee may interfere with the intestinal absorption of levothyroxine, though at a level less than eating bran [10]. Different substances cause other adverse effects that may be severe. Ketamine may cause hypertension and tachycardia and tricyclic and tetracyclic antidepressants increase its toxicity. On the other hand lithium can cause hyperthyroidism (but most often hypothyroidism) by affecting iodine metabolism of the thyroid itself and thus inhibits synthetic levothyroxine as well.

3.2.3 Review of Electrochemistry of Sodium Levothyroxine

Thyroxine (T4) (3, 5, 3’, 5’-tetraiodothyronine) [Scheme.3.1] is a derivative of the amino acid tyrosine & is unique in being the only iodine-containing compound of importance [11-15]. Thyroxine is an important biological component produced in the thyroid glands. The practical significances of thyroxine measurements for the diagnosis of hyperthyroidism and hypothyroidism have been known for many years.

The usual methods for the determination of T4 were enzyme immunoassays [16-18], time resolved fluorescence [19], radioimmunoassay (RIA) [20], capillary electrophoresis with laser-induced fluorescence [21], HPLC [22], chemiluminescence (CL) [23]. However these methods have some disadvantages such as expensive instrumentation and time consuming complicated operations. Electrochemical techniques have also been used for the detection of T4. Jacobsen & Fonahn [24] reported a DPP method for the determination of T4 at HMDE. Cathodic reduction of T4 on silver electrode was studied by Iwamoto & Co-workers [25] in comparison with its multi-step reduction at HMDE.

Surfactants are a kind of amphiphilic molecules with a polar (hydrophilic) head compatible with water on one side and a long hydrophobic tail compatible with oil on the other side. The applications of surfactants in electroanalytical chemistry have been widely reported. Surfactant modified carbon paste electrodes are used by Hu’s group for the determination of thyroxin in the presence of CTAB [26-28] and
also determination of thyroxine at the glassy carbon electrode modified with SWNTs was reported by F. Wang et al [29]. The results showed that the electrochemical response was greatly enhanced in the presence of trace surfactants.

3.3. Experimental

3.3.1. Apparatus

Electrochemical measurements were carried out with a model-201 electrochemical analyzer (EA-201 Chemilink system) in a conventional three-electrode system. The working electrode was a carbon paste electrode, having a cavity of 3mm diameter. A platinum wire and a saturated calomel electrode (SCE) were used as the counter and the reference electrode respectively.

3.3.2. Chemicals and Reagents.

T4 (obtained from Sigma) was dissolved in methanol with 2% of dilute orthophosphoric acid to prepare 0.5 mM standard stock solutions and stored at 4°C. SDS, CTAB and TX-100 were dissolved in water to form 1 μM solutions. Other chemicals used were of analytical grade except for spectroscopically pure graphite powder. All solutions were prepared with double distilled water.

3.3.3. Preparation of bare carbon paste electrode.

The carbon paste electrode was prepared by hand mixing 70% graphite powder and 30% silicon oil in an agate mortar for about 30 min to get homogeneous carbon paste. This carbon paste was then packed into the cavity of a Teflon tube electrode (3 mm in diameter). Before measurement the electrode was smoothened on a piece of transparent paper to get a uniform, smooth and fresh surface.

3.3.4. Preparation of surfactant modified carbon paste electrode.

Bare carbon paste electrode was prepared as explained in the section 3.3.3, and then the measured volumes of surfactant solutions were pipetted onto the surface of bare carbon paste electrode and kept quiet for about 5min. Then the electrode surface was carefully washed with distilled water to remove any unadsorbed particles and air dried. Then a stable and uniform surfactant immobilized film was formed on the surface of electrode and used for electrochemical analysis. To prepare surfactant mobilized carbon paste electrode, measured volume of surfactant solutions were
pipetted into an electrochemical cell along with the T₄ and the voltammograms were recorded in the potential range 0 mV to 1000 mV.

3.4. Results and Discussion

3.4.1. The voltammetric behaviour of sodium levothyroxine at carbon paste electrode

Fig. 3.1a shows the cyclic voltammogram of T₄ at carbon paste electrode, which was investigated in 0.1M HCl. When the potential initially sweeps from 0 to 1.0V a well-defined oxidation peak at 0.78V (O₁) in the positive scan and two reduction peaks at 0.53 (R₁) and 0.32V (R₂) on the reversal scan are observed in the first cycle. In the second and following cycles the peak currents of O₁ and R₁ decrease greatly and a new oxidation peak appears at about 0.48V (O₂). The peak currents of O₁ and R₁ decrease with the increasing of scan number while those of O₂ and R₂ remain stable. These results shows that the electrochemical behaviors of T₄ at carbon paste electrode are totally irreversible and that the products are strongly adsorbed on the electrode surface, blocking the mass transfer of T₄ from the solution to the electrode surface.

According to Murphy [30], the appearance of O₁ is due to the oxidation of OH on the phenol moiety of T₄. From the structure of T₄ it is clear that R₁ may be the reduction peak of iodine atoms on T₄. The O₂ and R₂ are the electrochemical responses of the product of T₄ produced from the oxidation of OH on T₄. (Although the proper electrochemical reactions involved in the oxidation/reduction of T₄ have been proposed, the hidden relationships between these responses are still unknown). When electrode potential was scanned over the range of 0.5-1.0V, the OH signal (i.e. O₁) is unchanged but no peaks were observed in the reverse scan. Such results prove that reduction of the iodine atoms on T₄ is achieved only after the oxidation of OH on T₄ because the iodine atoms on the phenol group of T₄ are activated after the stable benzene ring is destroyed during the oxidation process.

3.4.2. Electrochemical response of sodium levothyroxine at carbon paste electrode in presence of surfactants

It is well known that surfactants can be adsorbed on solid surfaces to form surfactant film [31, 32] which may alter the over voltage of the electrode and influence the rate of electron transfer. The electrochemical responses of T₄ at carbon
paste electrode in the presence of trace amount of surfactants onto the surface (immobilized form) as well as into the solution (mobilized form) were studied (Fig.3.2a to Fig.3.2f) in 0.1M HCl as supporting electrolyte with 100 mV/s scan rate. The low signal (solid line) is the cyclic voltammogram of T₄ in the absence of surfactants. However the voltammetric response is apparently improved (dotted line) in the presence of 10μL of SDS (Fig.3.2a and Fig.3.2b), CTAB (Fig.3.2c and Fig.3.2d) and TX-100 (Fig.3.2e and Fig.3.2f) both in mobilized and immobilized forms respectively. Comparative Cyclic voltammograms of 10μL TX-100, CTAB and SDS are also shown in the Fig.3.3a (a-d) and Fig.3.3b (a-d) for both mobilized and immobilized forms respectively. When the cationic surfactant CTAB and non-ionic surfactant TX-100 were used, there was increase in both oxidation as well as reduction peak currents both into the solution and onto the surface, shifting the cathodic peak potential to the negative side and anodic peak potential to more positive. But on the contrary when SDS is used large oxidation and reduction peak currents obtained. These results show that anionic surfactants can more effectively promote both oxidation as well as reduction of T₄. This may be because T₄ is an amphipathic molecule and in strong basic solution carboxyl group in T₄ is completely ionized and negatively charged. When cationic surfactant CTAB is added the adsorption of CTAB on the electrode surface may form a positively charged hydrophilic film on the electrode, therefore the oxidation of T₄ is facilitated by the cationic surfactant CTAB [25, 27]. This explains that T₄ exists in more positively charged by ionization in acidic media i.e. in orthophosphoric acid-methanol T₄ exists in cationic form and interacts with negative-charged head groups SDS via electrostatic interactions [33]. Therefore by preparing the Thyroxine in methanol with 2% orthophosphoric acid (pH 4) makes possible the enhancement of both oxidation as well as reduction peaks compared to CTAB and TX-100.

3.4.3. Effect of surfactant concentration on sodium levothyroxine

The effect of surfactant concentration on T₄ oxidation/reduction peak currents are shown in fig (Fig.3.4a and Fig.3.4b) for both mobilized and immobilized forms. The peak current increases linearly with the concentration of surfactant for all the three. The peak potential of oxidation peak (O₁) shifts towards positive side and the peak potentials of reduction peaks (R₁) & (R₂) tend to shift towards negative side in all the three cases. The current response for all the three surfactants in both mobilized
and immobilized forms were similar and in both the cases the enhancement in the current response for the SDS surfactant was more compared to CTAB and TX-100.

3.4.4. Effect of concentration of Hydrochloric acid

From the Fig.3.5 it is clear that as the concentration of HCl increased from 0.01M to 0.25M the anodic peak potential was decreased from 0.79 V for 0.01M to 0.77 V for 0.1M and again increased from 0.77 V for 0.1M to 0.785 for 0.25M. The oxidation was easier at 0.1M HCl, therefore 0.1M HCl was taken for further studies. The cathodic peak potentials of \( R_1 \) peak was negatively shifted and of \( R_2 \) positively shifted and the anodic peak potential of \( O_1 \) is negatively shifted. Multiple voltammograms indicate that the thyroxine got adsorbed on the surface of the electrode during the redox process. This conclusion is supported by linear nature of \( i_{pa} vs \nu \) plots [32] (Fig.3.6a).

3.4.5. Effect of scan rate

The dependence of peak current (\( i_{pa} \)) on the scan rate (\( \nu \)) was studied in the range of 50-300mV/s, a linear relationship was observed suggesting the adsorption-controlled process of the sodium levothyroxine (Fig.3.6a). The plot of \( i_{pa} vs \nu \) indicate an increase in peak current with an increase in sweep rate (Fig.3.6b) confirming that the electrode process at the electrode surface has some adsorption.

3.4.6. Effect of sodium levothyroxine concentration

The cyclic voltammetry showed successive enhancement of peak current on increasing \( T_4 \) concentration. The plot of peak current vs the respective concentration of \( T_4 \) was found to be linear in the range of \( 2\times10^{-4} \) M to \( 1.2\times10^{-3} \) M. The variation of anodic peak current (\( i_{pa} \)) with concentration shown in the fig (Fig.3.7a and Fig.3.7b).
3.5. Conclusion

- The present study has demonstrated the SDS, CTAB and TX-100 surfactants modified electrode by immobilization and mobilization methods for the determination of thyroxine in 0.1M HCl.
- The results show that anionic surfactant, SDS showed more current enhancement compared to cationic CTAB and non-ionic TX-100.
- This is because in acidic media T₄ exists in cationic form and interacts with negatively charged SDS through electrostatic interactions.
- Based on the different existing forms of T₄ (cationic or anionic) depending on the preparation media, suitable ionic surfactants can be used to improve the sensitivity of determination of T₄ using a carbon paste electrode.
- Due to good sensitivity, selectivity and reliability of the modified electrode, there is a good possibility of extending the proposed method to the analysis of thyroxine in real biological samples.
- With its low cost and ease of preparation the SDS surfactant modified CPE seems to be of great utility for further sensor development.
Scheme 3.1. Structure of sodium levothyroxine

Figure 3.1a. Cyclic voltammogram of 1×10⁻⁴ M Thyroxine at CPE in 0.1M HCl; scan rate, 100mV/s.
Figure 3.1b. Appearance of new peak @ around 0.48V after the first scan in the electrochemical response of $1 \times 10^{-4}$ M Thyroxine in 0.1M HCl; scan rate, 100mV/s

Figure 3.2a. Cyclic voltammogram $1 \times 10^{-4}$ M Thyroxine at the bare CPE (solid line) and, $1 \times 10^{-5}$ M SDS surfactant mobilized CPE (dotted line).
Figure 3.2b. Cyclic voltammogram $1 \times 10^{-4}$ M Thyroxine at the bare CPE (solid line) and $1 \times 10^{-5}$ M SDS surfactant immobilized CPE (dotted line).

Figure 3.2c. Cyclic voltammogram $1 \times 10^{-4}$ M Thyroxine at the bare CPE (solid line) and $1 \times 10^{-5}$ M CTAB surfactant mobilized CPE (dotted line).
Figure 3.2d. Cyclic voltammogram $1 \times 10^{-4}$ M Thyroxine at the bare CPE (solid line) and $1 \times 10^{-5}$ M CTAB surfactant immobilized CPE (dotted line).

Figure 3.2e. Cyclic voltammogram $1 \times 10^{-4}$ M Thyroxine at the bare CPE (solid line) and $1 \times 10^{-5}$ M TX-100 surfactant mobilized CPE (dotted line).
Figure 3.2f. Cyclic voltammogram $1 \times 10^{-4}$ M Thyroxine at the bare CPE (solid line) and $1 \times 10^{-5}$M TX-100 surfactant immobilized CPE (dotted line).

Figure 3.3a. Cyclic voltammograms for $1 \times 10^{-4}$ M Thyroxine at (a-d). (a) is bare CPE, (b) is $1 \times 10^{-5}$M TX-100, (c) is $1 \times 10^{-5}$M CTAB and (d) is $1 \times 10^{-5}$M SDS surfactants mobilized CPE.
Figure 3.3b. Cyclic voltammograms for $1\times10^{-4}$ M Thyroxine at (a-d). (a) bare CPE and (b) $1\times10^{-5}$ M TX-100, (c) $1\times10^{-5}$ M CTAB and (d) $1\times10^{-5}$ M SDS surfactants immobilized CPE.

Figure 3.4a. Effect of surfactant concentration variation on Thyroxine oxidation peak current (a-c); (a) $1\times10^{-5}$ M TX-100, (b) $1\times10^{-5}$ M SDS and (c) $1\times10^{-5}$ M CTAB at 0μM, 2μM, 4μM, 6μM, 8μM, 10μM mobilized CPE.
Figure 3.4b. Effect of surfactant concentration variation on Thyroxine oxidation peak current (a-c); (a) $1 \times 10^{-5}$ M TX-100, (b) $1 \times 10^{-5}$ M SDS and (d) $1 \times 10^{-5}$ M CTAB at 0 µM, 2 µM, 4 µM, 6 µM, 8 µM, 10 µM immobilized CPE.

Figure 3.5. Graph of the different concentration of Hydrochloric acid (a-f; 0.01 M, 0.05 M, 0.1 M, 0.15 M, 0.2 M and 0.25 M).
Figure 3.6a. Graph of current vs scan rate.

Figure 3.6b. Cyclic voltammograms of different scan rates (a-f; 50mV/s, 100mV/s, 150mV/s, 200mV/s, 250mV/s and 300mV/s).
Figure 3.7a. Cyclic voltammograms for the different concentration of T4 (a-g; $2 \times 10^{-4}$ M, $3 \times 10^{-4}$ M, $4 \times 10^{-4}$ M, $6 \times 10^{-4}$ M, $8 \times 10^{-4}$ M, $1 \times 10^{-3}$ M and $1.2 \times 10^{-3}$ M).

Figure 3.7b. Graph of different concentration of T4 (a-f; $2 \times 10^{-4}$ M, $4 \times 10^{-4}$ M, $6 \times 10^{-4}$ M, $8 \times 10^{-4}$ M and $1.2 \times 10^{-3}$ M).
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3.6. References


PART-B

ELECTROCATALYTIC OXIDATION OF SODIUM LEVOTHYROXINE WITH PHENYL HYDRAZINE AS A MEDIATOR AT CARBON PASTE ELECTRODE: A CYCLIC VOLTAMMETRIC STUDY


Sodium levothyroxine
3.7. Introduction

In this chapter the electrochemical oxidation of sodium levothyroxine (T₄) has been studied on carbon paste electrode (CPE) with phenyl hydrazine homogenous as mediator. The discussion involves the chemistry, biological relevance of sodium levothyroxine and its oxidation behavior in 0.1 M HCl solution at the bare and modified carbon paste electrode. The effect of scan rate and concentration of sodium levothyroxine in presence of trace phenyl hydrazine was studied. The practical application of the phenyl hydrazine mediated CPE in the determination of T₄ in a commercial tablet sample demonstrated that it has good selectivity and high sensitivity. Thus this method offers a promising, relatively uncomplicated protocol for the preparation of biosensors.

3.8. Chemistry and Biological Relevance of Sodium Levothyroxine

The chemistry and biological relevance of sodium levothyroxine has been explained in detail in chapter 3A section 3.2.

3.9. Review of Cyclic Voltammetry of Sodium Levothyroxine.

The design, fabrication and application of sensitive and selective electrochemical sensors have been of considerable interests in recent years. The electrochemical sensors based on chemically modified electrodes (CMEs) have widely been used for the detection of biologically important organic compounds. The modified electrodes using transition metal complexes [1], organic electron mediators [2] and polymer-modified electrodes [3] have attracted the most attention in this regard. One of the most important characteristics of the electron mediators, which are used in modified electrodes, is sufficient sensitivity and selectivity [4].

Phenyl hydrazine was the first hydrazine derivative characterized, reported by Emil-Fischer in 1875. Hydrazine’s are nitrogen-containing compounds and constitute an important class of xenobiotic agents occurring in natural organisms and the free electron pairs of the nitrogen from the amino group, which are involved in charge transfer phenomena, as a support for the electron conduction through biostructured systems [5, 6].

As told in the previous chapter 3A section 3.2.3 the usual methods for the determination of T₄ were immunoassays, high performance liquid chromatography (HPLC). However these methods have some disadvantages such as expensive
instrumentation and time consuming, complicated operations. Some electrochemical techniques have also been used for the detection of T4 which minimize the sample pre-treatment; reduce the cost and time of analysis [7-9].

3.10. Experimental
3.10.1. Apparatus

Electrochemical measurements were carried out with a model-201 electrochemical analyzer (EA-201 Chemilink system) in a conventional three-electrode system. The working electrode was a carbon paste electrode, having cavity of 3 mm diameter. The counter electrode was a bright platinum wire with a saturated calomel electrode (SCE) completing the circuit.

3.10.2. Chemicals and Reagents.

T4 (obtained from Sigma, >99.0%) was dissolved in methanol with 2% of dilute orthophosphoric acid to prepare 0.5 mM standard stock solutions and stored at 4 °C. Phenyl hydrazine (>97%) was prepared by using double distilled water. Other chemicals used were of analytical grade except for spectroscopically pure graphite powder. All solutions were prepared with double distilled water.

3.10.3. Preparation of bare carbon paste electrode

The preparation of bare carbon paste electrode has been explained in detail in chapter 3A section 3.3.3.

3.10.4. Preparation of phenyl hydrazine modified carbon paste electrode

Measured volume of 0.1M HCl and phenyl hydrazine solution were pipetted into an electrochemical cell. Then the standard solution of T4 was added to the cell. The voltammograms were recorded for T4 in the potential range of 0.0 V – 1.0 V.

3.11. Results and Discussions
3.11.1. Electrocatalytic oxidation of sodium levothyroxine at carbon paste electrode

Experimental results show that phenyl hydrazine acts as a suitable intermediate for electron transfer in the oxidation of T4 at the surface of carbon paste electrode. Fig.3.8a shows the cyclic voltammetric responses of 0.1 mM phenyl...
hydrazine (curve a) and T₄ in the absence (curve b) and in the presence (curve c) of phenyl hydrazine in 0.1 M HCl as supporting electrolyte. T₄ oxidation peak current of (O₁) increases sharply in the presence of phenyl hydrazine.

In the absence of phenyl hydrazine, a well-defined oxidation peak appears at 780 mV (O₁) in the positive scan when the potential initially sweeps from 0.0 mV to 1000 mV and two indiscernible reduction peaks (R₁ and R₂) at 520 mV and 330 mV are obtained on the reversal scan (Fig. 3.8b). However the peak currents of O₁ decrease greatly and another oxidation peak (O₂) at about 420 mV appears on the second scan. During following successive cyclic scans, the peak O₁ decrease all the same with the increasing of scan number, resulting from the fact that electrode surface is blocked by the strong adsorption of the reaction products. When the electrode potential was scanned over the range of 500 – 1000 mV, the O₁ signal is unchanged but no peaks were observed in the reverse scan. All results show that electrochemical oxidation of T₄ is a totally irreversible process, which can be explained by the strong adsorption of reduction products of T₄ at the electrode surface. The peaks O₂ and R₂ are ascribed to the electrochemical responses of the product of T₄ [10]. According to Iwamoto et al.'s report [11] R₂ and O₂ are attributed to the reduction and the oxidation of the iodine atoms on T₄ respectively, and O₂ always appears following the R₂. As for the oxidation peak O₁ is considered it may be caused by the oxidation of the phenolic hydroxyl group on the T₄ molecule and the R₁ is the reduction response of the products of T₄ such as the hydroquinone–benzoquinone redox system produced from the oxidation of phenolic hydroxyl group on T₄ [12].

Fig. 3.8a shows the electrochemical responses of T₄ with phenyl hydrazine blank (curve a), in the absence (curve b) and in the presence (curve c) of phenyl hydrazine at CPE. It is clear from the fig. 3.8a that, the anodic peak current of (O₁) of T₄ in the presence of phenyl hydrazine is much enhanced than at the bare CPE. Also the oxidation peak potential of T₄ in the presence of phenyl hydrazine shifts slightly from 780 mV to 800 mV. The anodic peak current difference (Iₚₐ) in the presence and absence of phenyl hydrazine shows that phenyl hydrazine acts as a suitable intermediate for electron transfer in the oxidation of T₄.

The main difficulty in determining the exact mechanism is identification of the intermediate in the oxidation process [13]. The hydrazines are easier to oxidize so
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their oxidation behaviors were studied, and the result shows that the oxidation process is based on the hydrazine moiety and not on their derivative groups [14-18].

The proposed sequence of reactions that occur between phenyl hydrazine and T₄ at carbon paste electrode is shown below.

Phenylhydrazine \( (aq) \) \( \rightarrow \) (Phenylhydrazine) \( \text{ox} (aq) \) 
(Phenylhydrazine) \( \text{ox} (aq) \) + Sodium levothyroxine \( (aq) \) \( \rightarrow \) Phenylhydrazine 
(Phenylhydrazine) \( (aq) \) + (Sodium levothyroxine) \( \text{ox} (aq) \)

The above sequence of reactions between phenyl hydrazine and T₄ can be explained as follows. First phenyl hydrazine undergoes oxidation to diazenyl benzene as shown in the step 1 of scheme 3.2 and attach to the electrode surface as shown in step 1 of scheme 3.3 followed by two electron transfer to the electrode and the oxidized phenyl hydrazine then helps the T₄ to undergo oxidation probably either one of the way (2a or 2b) as shown in scheme 3.3.

But when the system contains methanol (since the dilution media for sodium levothyroxine is methanol) then there is a chance of formation of methoxy benzene, hydrazine with dinitrogen as the leaving group in small amounts giving the overall reaction as shown in scheme 3.2 [19-22].

3.11.2. Effect of phenyl hydrazine concentration.

The effect of phenyl hydrazine concentration on the anodic peak current was studied for the range of 0.025—0.2 mM phenyl hydrazine concentration, in the solutions containing 0.1 mM T₄ in 0.1 M HCl was shown in Fig. 3.9a and Fig. 3.9b. The results showed that by increasing phenyl hydrazine concentration up to 0.1 mM the anodic peak current increased, whereas higher concentration of phenyl hydrazine caused a slight decrease in the peak current and almost keeps unchangeable. This may be due to the fact that the adsorption of phenyl hydrazine at the carbon paste electrode surface tends to saturation due to the formation of phenyl hydrazine aggregations. Therefore 0.1 mM was selected as the optimal mediator concentration. The peak potential for the system shifts slightly to a more positive potential.

3.11.3. Effect of sodium levothyroxine concentration.

The cyclic voltammogram showed successive enhancement of anodic peak current with increase in concentration of T₄. The variation of peak current \( (I_{pa}) \) with T₄ concentration (Fig. 3.10) in presence of 0.1 mM phenyl hydrazine was linear in
the range of 0.025—0.1 mM with a correlation coefficient 0.997. It was also observed that the anodic peak potential ($E_{pa}$) was shifted towards positive potential with increasing concentration showing adsorption of the oxidized product over the electrode surface. The detection limit of $T_4$ in the presence of 0.1 mM phenyl hydrazine was found to be 2.5 $\mu$M by cyclic voltammetric method.

3.11.4. Effect of scan rate

The effect of the potential scan rate on the electrocatalytic properties of phenyl hydrazine in a 0.1 M HCl supporting electrolyte containing 0.1 mM $T_4$ was studied. The obtained results showed that the anodic peak current increased linearly with the increase of scan rate in the range of 100-350 mV/s, it seems that the electrode process is controlled by adsorption. This is consistent with the discussion above, i.e. the decrease of the peak current of $O_1$ with increase of scan numbers.

The dependence of the oxidation peak current ($I_{pa}$) as well as peak current function ($I_{pa}/v^{1/2}$) and also peak potential on the scan rate ($v$) were studied in the range 100-350 mV/s as shown in figs.3.11a to 3.11c. A linear relationship was observed between $\log I_{pa}$ and $\log v$ with a correlation coefficient of 0.990 (Fig.3.11a). The plot of $I_{pa}/v^{1/2}$ vs. $\log v$ indicated an increase in peak current with an increase in sweep rate (Fig.3.11b) confirming that the electrode process at the electrode surface has some adsorption. Also, the plot of peak potential $E_{pa}$ vs. $\log v$ (Fig.3.11c) was linear with a correlation coefficient of 0.998. Fig.3.11c shows the relationship between the oxidation peak potential $E_{pa}$ and the $\log v$ and can be expressed by the following equation:

$$E_{pa} = 0.1329 \log v + 0.535 \quad (R=0.998) \quad (3.1)$$

It can be noted from Fig.3.11c that, along with an increase in the scan rate, the peak potential for the catalytic oxidation of $T_4$ shifts to the more positive potentials, suggesting a kinetic limitation to the reaction between the phenyl hydrazine and $T_4$.

The values of $\alpha_{an}$ (where $\alpha_{an}$ is the charge transfer coefficient) were calculated for the oxidation peak of $T_4$ in 0.1 M HCl in the presence and absence of phenyl hydrazine mediator at CPE, according to the following equation [23].

$$\alpha_{an} = 0.0477 / (E_{pa}-E_{pa}/2) \quad (3.2)$$

The values for $\alpha_{an}$ were found to be 0.91 and 0.64 for the oxidation of $T_4$ at CPE in the presence and absence of phenyl hydrazine respectively. These results clearly
show that the rate of the electron-transfer process is greatly enhanced in presence of mediator. This phenomenon is thus confirmed by large $I_{pa}$ values recorded at the CPE in presence of phenyl hydrazine.

3.11.5. Analytical application

In order to evaluate the applicability of proposed method, $T_4$ was determined in the commercially available Eltroxine IP tablets (declared content is 100 mcg of $T_4$ in one tablet). The average mass of 10 tablets were weighed accurately and finely powdered and transferred to a 50 ml volumetric flask and dissolved in methanol. The mixture was sonicated for 30 min and it was then filtered. After that a suitable aliquot of the clear filtrate was quantitatively diluted with 0.1 M HCl solution and the determination of sodium levothyroxine in tablets was carried out by applying a calibration plot. A typical cyclic voltammogram for the determination of $T_4$ in the commercial Eltroxine tablets is as shown in the fig.3.12. $T_4$ in commercial Eltroxine IP tablets obtained from cyclic voltammetric determination are presented in Table.3.1. The results were satisfactory, showing that the proposed method could be efficiently used for the determination of $T_4$ in pharmaceutical preparations.
3.12. Conclusion

- This is a new cyclic voltammetric method approach for the determination of T₄ using phenyl hydrazine as the mediator.
- The electrochemical oxidation of T₄ at carbon paste electrode showed that the oxidation peak current of T₄ was improved in the presence of phenyl hydrazine.
- In the presence of methanol with dilute orthophosphoric acid as the preparation medium for T₄, the oxidation peak was more selective for the determination of T₄.
- The electrochemical response is adsorption controlled and irreversible in nature.
- The oxidation peak current of T₄ was linear in range 0.025—0.1 mM, with a detection limit of 2.5 μM. The proposed method has been practically and successfully applied for the determination of T₄ in commercial tablets.
- The proposed method could be efficiently used for the determination of T₄ in pharmaceutical preparations.
Fig. 3.8a. Cyclic voltammograms of 0.1mM phenyl hydrazine (curve a) and 0.1mM Sodium Levothyroxine in the absence (b) and presence (c) of 0.1mM phenyl hydrazine at a carbon paste electrode in 0.1M HCl with a scan rate of 100 mV/s, in the potential range 0.0mV to 1000mV.

Fig. 3.8b. Appearance of new peak at around 480 mV (dashed line) after the first scan in the electrochemical response of 0.1 mM Sodium Levothyroxine in 0.1M HCl; scan rate, 100mV/s.
Fig. 3.9a. Cyclic voltammograms for 0.1 mM Sodium levothyroxine at different concentrations of phenyl hydrazine (a-e). (a) bare CPE, (b) 0.025, (c) 0.05, (d) 0.075 and (e) 0.1 mM phenyl hydrazine.

Fig. 3.9b. Plot of different concentrations of phenyl hydrazine v/s anodic peak current in the presence of 0.1 mM sodium levothyroxine.
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Fig. 3.10. Plot of concentration of sodium levothyroxine vs anodic peak current in the presence of 0.1 mM phenyl hydrazine.

Fig. 3.11a. Dependence of log \( I_{pa} \) on log \( v \) in the presence of 0.1 mM Sodium levothyroxine and 0.1 mM phenyl hydrazine.
Fig. 3.11b. Dependence of $I_{pa}/v^{1/2}$ on log $v$ in the presence of 0.1 mM Sodium levothyroxine and 0.1 mM phenyl hydrazine.

Fig. 3.11c. Dependence of $E_{pa}$ on log $v$ in the presence of 0.1 mM Sodium levothyroxine and 0.1 mM phenyl hydrazine.
Fig. 12. Typical cyclic voltammograms for the determination of sodium levothyroxine in a commercial tablet sample in the absence (curve a) and in the presence (curve b) of 0.1mM phenyl hydrazine at a carbon paste electrode with a scan rate of 100 mV/s.

Scheme. 3.2. Proposed mechanism of phenyl hydrazine.
Scheme 3.3. Probable mechanism of phenyl hydrazine with Sodium levothyroxine.

<table>
<thead>
<tr>
<th>Sample No</th>
<th>Specified (mcg/tab)</th>
<th>Detected (mcg/tab)</th>
<th>RSD% (n=3)</th>
</tr>
</thead>
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<tr>
<td>1</td>
<td>100</td>
<td>98</td>
<td>1.94</td>
</tr>
<tr>
<td>2</td>
<td>100</td>
<td>99</td>
<td>1.75</td>
</tr>
<tr>
<td>3</td>
<td>100</td>
<td>96</td>
<td>1.5</td>
</tr>
</tbody>
</table>

Table 3.1. Determination of sodium levothyroxine in the commercial Eltroxine IP tablets
3.13. References


